10/049502

C of C

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313 on April 6, 2007.

Glenn P. Ladwig

Glenn P. Ladwig, Patent Attorney

REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.322
Docket No. USF-T144X
Patent No. 7,157,438

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| Applicant | : | Said M. Sebti |
| Issued | : | January 2, 2007 |
| Patent No. | : | 7,157,438 |
| For | : | RhoB as a Suppressor of Cancer Cell Growth and Cell Transformation |

ATTN: Certificate of Correction Branch
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450



REQUEST FOR CERTIFICATE OF CORRECTION UNDER 37 C.F.R. § 1.322 (OFFICE MISTAKE)

Sir:

A Certificate of Correction (in duplicate) for the above-identified patent has been prepared and is attached hereto.

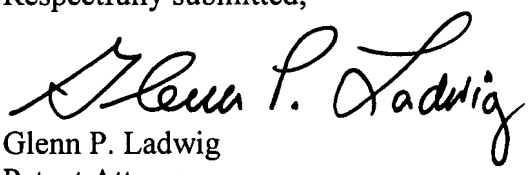
In the left-hand column below is the column and line number where errors occurred in the patent. In the right-hand column is the page and line number in the application where the correct information appears.

| **Patent Reads:** | **Application Reads:** |
|---|---|
| Column 2, lines 19-20: | Page 2, line 24 to page 3, line 1: |
| "predicted to prefers a leucine. Ras proteins" | --predicted to be differ.<br>Low molecular weight GTPases require prenylation, a lipid posttranslational modification, for their biological activity. Zhang, F.L. et al., *Annu. Rev. Biochem.*, 65:241-269, (1996). The two enzymes that catalyze these modifications for Ras and Rho GTPases are farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I). The enzymes recognize the carboxyl terminal sequence CAAX (C=cysteine, A=aliphatic amino acid and X=any amino acid) and covalently attach a farnesyl or a geranylgeranyl to the cysteine. FTase prefers a methionine or a serine at the X position whereas GGTase I prefers a leucine. Ras proteins-- |
| Column 5, line 32: | Page 7, line 21: |
| "or transferring receptor" | --or transferrin receptor-- |
| Column 10, line 15: | Page 14, line 17: |
| "show that RhoB-P" | --show that RhoB-F-- |

True and correct copies of pages 2, 3, 7, and 14 of the specification as filed which support the applicant's assertion of the errors on the part of the Patent Office accompanies this Certificate of Correction.

Approval of the Certificate of Correction is respectfully requested.

Respectfully submitted,



Glenn P. Ladwig
Patent Attorney
Registration No. 46,853
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

GPL/gyl/mv

Attachments: Copy of pages 2, 3, 7, and 14 of the specification
Certificate of Correction in duplicate

# UNITED STATES PATENT AND TRADEMARK OFFICE
# CERTIFICATE OF CORRECTION

PATENT NO. : 7,157,438
APPLICATION NO. : 10/049,502
ISSUE DATE : January 2, 2007
INVENTOR(S) : Said M. Sebti



It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2
Lines 19-20, "predicted to prefers a leucine. Ras proteins" should read
--predicted to be differ.

Low molecular weight GTPases require prenylation, a lipid posttranslational modification, for their biological activity. Zhang, F.L. et al., *Annu. Rev. Biochem.*, 65:241-269, (1996). The two enzymes that catalyze these modifications for Ras and Rho GTPases are farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I). The enzymes recognize the carboxyl terminal sequence CAAX (C=cysteine, A=aliphatic amino acid and X=any amino acid) and covalently attach a farnesyl or a geranylgeranyl to the cysteine. FTase prefers a methionine or a serine at the X position whereas GGTase I prefers a leucine. Ras proteins--.

Column 5
Line 32, "or transferring receptor" should read --or transferrin receptor--.

Column 10
Line 15, "show that RhoB-P" should read --show that RhoB-F--.

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

# UNITED STATES PATENT AND TRADEMARK OFFICE
# CERTIFICATE OF CORRECTION

PATENT NO. : 7,157,438
APPLICATION NO. : 10/049,502
ISSUE DATE : January 2, 2007
INVENTOR(S) : Said M. Sebti



It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2
Lines 19-20, "predicted to prefers a leucine. Ras proteins" should read
--predicted to be differ.

Low molecular weight GTPases require prenylation, a lipid posttranslational modification, for their biological activity. Zhang, F.L. et al., *Annu. Rev. Biochem.*, 65:241-269, (1996). The two enzymes that catalyze these modifications for Ras and Rho GTPases are farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I). The enzymes recognize the carboxyl terminal sequence CAAX (C=cysteine, A=aliphatic amino acid and X=any amino acid) and covalently attach a farnesyl or a geranylgeranyl to the cysteine. FTase prefers a methionine or a serine at the X position whereas GGTase I prefers a leucine. Ras proteins--.

Column 5
Line 32, "or transferring receptor" should read --or transferrin receptor--.

Column 10
Line 15, "show that RhoB-P" should read --show that RhoB-F--.

MAILING ADDRESS OF SENDER:
Saliwanchik, Lloyd & Saliwanchik
P.O. Box 142950
Gainesville, FL 32614-2950

Ras is mutated to a GTPase-deficient form that leads to constitutive activation of the above signaling pathways, uncontrolled proliferation, and survival of human tumors. Barbacid, M. *Annu.Rev.Biochem.* 56: 779-827, (1987); and Bos, J.L. *Cancer Res.* 49: 4682-4689, (1989). Family members (closely related) to Ras, such as RhoA and Rac1, have also been shown to be intimately involved in proliferation and transformation. For example, both RhoA and Rac1 are required for the G1 to S phase transition during the cell division cycle. Olson, M.F., et al., A. *Science* 269: 1270-1272, (1995). Furthermore, GTP-locked RhoA and Rac1 are transforming, and dominant negative forms of these GTPases inhibit Ras-induced malignant transformation. Khosravi-Far, R., et al., *Mol. Cell. Biol.* **15**: 6443-6453, (1995) and Qiu, R.Get al., *Nature* **374**: 457-459 (1995). Unlike RhoA and Rac1, less is known about the involvement of RhoB GTPase in proliferation and transformation. There are several features that distinguish RhoB from other Rho proteins. Firstly, its cellular localization within early endosomes and the pre-lysosomal compartment is different from the localization of other members. Mellow, H. et al., *J. Biol. Chem.* **273**: 4811-4814, (1998). Secondly, RhoB is an immediate early response gene that is induced by PDGF, transforming growth factor-α, the non-receptor tyrosine kinase v-src, and ultraviolet irradiation. Jahner, D. et al., *Mol. Cell. Biol.* **11**: 3682-3690, (1991), and Fritz, G., et al., *J. Biol. Chem.* **270**: 25172-25177, (1995). However, these studies were mostly performed using fibroblasts, and whether RhoB is also an immediate early response gene in human cancer cells of epithelial origin is not known. Third and finally, RhoB mRNA and RhoB protein levels turn over much more rapidly (with half-lives of 20 and 120 min, respectively) than other GTPases, which typically have half lives on the order of 24 hrs. Therefore, although RhoA and RhoB share 90% amino acid sequence homology, their physiological functions are predicted to be differ.

Low molecular weight GTPases require prenylation, a lipid posttranslational modification, for their biological activity. Zhang, F.L., et al., *Annu. Rev. Biochem.* 65: 241-269, (1996). The two enzymes that catalyze these modifications for Ras and Rho GTPases are farnesyltransferase (FTase) and geranylgeranyltransferase I (GGTase I). The enzymes recognize the carboxyl terminal sequence CAAX (C=cysteine, A=aliphatic amino acid and X=any amino acid) and covalently attach a farnesyl or a geranylgeranyl to the cysteine. FTase prefers a methionine or a serine at the X position whereas GGTase I

prefers a leucine. Ras proteins (e.g. H-, K- and N-Ras) are farnesylated, whereas RhoA and Rac1 are geranylgeranylated. Although RhoB has a C-terminal leucine, which would be predicted to dictate only geranylgeranylation, it is both farnesylated and geranylgeranylated in cells. Because Ras is constitutively activated in 30% of human cancers and Ras farnesylation is required for its malignant transforming activity, Lebowitz, P.F., et al., *J. Biol. Chem.* **272**: 15591-15594, (1997), FTase inhibitors (FTIs) were designed as novel anticancer drugs. FTIs have shown impressive antitumor activity and lack of toxicity in preclinical models and are presently in various human clinical trial phases. Sebti, S.M., et al., *Pharmacol. Ther.* **74**: 103-114, (1997); Gibbs, J.B., et al., *Annu. Rev. Pharmacol. Toxicol.* **37**: 143-166, (1997); and Cox, A.D., et al., *Biochim. Biophys. Acta* **1333**: F51-F71, (1997). Although FTIs were initially hypothesized to inhibit tumor growth by targeting Ras, recent evidence suggests that other farnesylated proteins may be involve, Lebowitz, P.F., et al., *Oncogene* **17**: 1439-1445, (1998).

RhoB has been suggested as a potential candidate for several reasons. Firstly it is a substrate for FTase and FTIs inhibit its farnesylation, resulting in decreased RhoB-F and increased RhoB-GG. Secondly, RhoB's short half life more closely resembles the kinetics of FTIs reversal of transformation than Ras. Third, a RhoB/RhoA chimeric protein that is exclusively geranylgeranylated is growth inhibitory. Thereby, a myristylated form of RhoB which is not prenylated prevents FTIs from inhibiting Ras transformation. However, the biochemical properties of myristylated RhoB are not the same as wild type RhoB making it difficult to interpret the data. Furthermore, RhoB has been shown to be farnesylated by FTase as well as by GGTase I. Fourth and final, most ~~of~~ the studies carried out so far used murine fibroblasts. Therefore, although there is some evidence suggesting RhoB's involvement in FTIs antitumor activity, direct evidence implicating RhoB in FTIs mechanism of action in human tumors is lacking.

A novel function for RhoB(WT) as a potent inhibitor of malignant transformation and a suppressor of human tumor growth is disclosed herein. Furthermore, both RhoB-F and RhoB-GG induce apoptosis, inhibit oncogenic signaling and suppress transformation *in vitro* and *in vivo*. These findings demonstrate the tumor suppressing activity of RhoB, and strongly suggest that, contrary to prior suggestions, RhoB-F is not a target for FTIs in human cancer cells.

In one aspect, the method of the present invention is performed by introducing a nucleic acid construct encoding the RhoB protein or a variant thereof into the cell, whereby the RhoB protein, or variant thereof, is made within the cell from the construct. "Nucleic acid construct" refers to an assembly which is capable of directing the expression of the sequence(s) or gene(s) of interest. The construct preferably includes transcriptional promoter/enhancer or locus defining element(s), or other elements which control gene expression by other means such as alternate splicing, nuclear RNA export, post-translational modification of messenger, or post-transcriptional modification of protein. In addition, the construct preferably includes a sequence which, when transcribed, is operably linked to the sequence(s) or gene(s) of interest and acts as a translation initiation sequence. Optionally, the vector construct may also include a signal which directs polyadenylation, a selectable marker such as Neo, TK, hygromycin, phleomycin, histidinol, or DHFR, as well as one or more restriction sites and a translation termination sequence. In addition, if the vector construct is placed into a retrovirus, the vector construct preferably includes a packaging signal, long terminal repeats (LTRs), and positive and negative strand primer binding sites appropriate to the retrovirus used (if these are not already present).

Within one embodiment, a recombinant viral vector (preferably, but not necessarily, a recombinant MLV retrovirus) carries a vector construct containing a RhoB or variant RhoB gene expressed from an event-specific promoter, such as a cell cycle-dependent promoter (e.g., human cellular thymidine kinase or transferrin receptor promoters), which will be transcriptionally active primarily in rapidly proliferating cells, such as tumors. In this manner, rapidly replicating cells which contain factors capable of activating transcription from these promoters are preferentially destroyed by the cytotoxic agent produced by the vector construct.

Administration of RhoB as a salt may be appropriate. Examples of pharmaceutically acceptable salts are organic acid addition salts formed with acids which form a physiological acceptable anion, for example, tosylate, methanesulfonate, acetate, citrate, malonate, tartarate, succinate, benzoate, ascorbate, alpha-ketoglutarate, and alpha-glycerophosphate. Suitable inorganic salts may also be formed, including hydrochloride, sulfate, nitrate, bicarbonate, and carbonate salts.

RhoB-F and RhoB-GG, CAAX box mutants of RhoB are generated that are either exclusively farnesylated or geranylgeranylated. To illustrate the effects of the RhoB mutants on transformation of human cancer cells, both *in vitro* and *in vivo* assays are used. The effects of the RhoB mutants on the ability of human cancer cells to grow foci in a focus formation assay are first shown. Several human cancer cell lines are transfected with the RhoB mutant DNAs and the foci that formed are scored 14 days later. Control transfections are also performed with the tumor suppressor p53 and the GTPase RhoA, a closely related RhoB family member that shares 90% amino acid homology. Figure 1A and Table 1 show that the human pancreatic cancer cell line, Panc-1, transfected with empty vector DNA, pCMV, forms numerous foci (155-233 foci). In contrast, Panc-1 cells transfected with wild type RhoB grow only 23-39 foci. Furthermore, RhoA-transfected Panc-1 cells grow more foci (over 346-409) than pcDNA3 empty vector-transfected cells (163-211 foci) (Figure 1A and Table 1). In contrast, Panc-1 cells transfected with p53 far fewer foci (21-27 foci). This illustrates that whereas RhoA promotes, RhoB suppresses foci formation, and thus RhoB is capable of antagonizing transformation of Panc-1 cells. The prenylation status of RhoB does not affect its ability to inhibit foci formation of Panc-1 cells. Figure 1A and Table 1 show that RhoB-F and RhoB-GG are also potent inhibitors of Panc-1 foci formation. Whether this inhibition of foci formation by RhoB can be extended to human cancer cells without Ras mutations is now determined. Table 1 shows that RhoB-F, RhoB-GG and RhoB-WT are potent inhibitors of foci formation of C33A and Hela (cervical carcinomas), and Saos-2 (osteosarcoma) none of which express mutated Ras. In all of these cell lines, p53 inhibited whereas RhoA promoted foci formation (Table 1).

Similar results are obtained using A549 lung carcinoma cells in place of Panc-1 cells.

**Both RhoB-F and RhoB-GG as well as RhoB-WT inhibit anchorage-independent and anchorage–dependent growth of Panc-1 cells.** To illustrate the effects of the RhoB mutants on malignant transformation, Panc-1 or A549 cells are transfected with the above constructs and several stable clones are isolated. Expression of RhoB is controlled by Western blotting using several clones picked from each construction (Figure 1B). Representative clones are selected for further studies. The clones picked for further study are RhoB-WT clone 2 (W2), RhoB-F clone 2 (F2) and Rho-GG clone 2 (G2) (Figure 1B).